

Reduced proteasome activity in the aging brain results in ribosome stoichiometry loss and aggregation

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Editor: Maria Polychronidou

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

The reviewers' comments and authors' responses are not available with this article, as the initial review process took place with another journal.

Thank you again for submitting your work to Molecular Systems Biology. The reviews from the other journal were quite constructive so as we discussed previously, we decided to use these reports rather than reviewing the study from scratch. Thank you for the detailed and really well-structured point by point response, it made it easy for me to evaluate the changes. I feel that the responses to the reviewers' comments seem to satisfactorily address the points raised. As most of the reviewers' concerns referred to the need to provide further support for the main conclusions, but there were no serious concerns on the technical aspects of the core experiments reported in the study, we have decided to proceed with making a decision based on our evaluation of the study and your responses.

Overall, we think that the in vivo proteasome inhibitor experiments provide additional support for the proposed role of the proteasome. Similarly the MS analysis of aggregates from young vs old mouse brains and the immunostaining in fish brain samples provide further support for the related conclusions. Several other comments referred to the need to include clarifications, text edits, methodological details and statistical support and we think that they have been satisfactorily addressed.

In summary, we think that the explanations and additional analyses performed in response to the reviewers' comments are satisfactory. As such, we have decided to proceed with the publication of your study in Molecular Systems Biology, pending some minor revisions listed below:

The Authors have made the requested editorial changes.

Thank you for sending us your revised manuscript. Before we can accept the manuscript for publication I would ask you to fix a few remaining minor issues listed below:

The Authors have made the requested editorial changes.

Thank you again for sending us your revised manuscript. We are now satisfied with the modifications made and I am pleased to inform you that your paper has been accepted for publication.

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

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Journal Submitted to: MSB

Manuscript Number: MSB-20-9596

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner;
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way;
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if $n < 5$, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
 - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ^2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
 - are tests one-sided or two-sided?
 - are there adjustments for multiple comparisons?
 - exact statistical test results, e.g., P values = x but not P values < x;
 - definition of 'center values' as median or average;
 - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

USEFUL LINKS FOR COMPLETING THIS FORM

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1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	For proteomic analysis, sample size was chosen on the basis of prior RNAseq experiments that analysed similar samples (Baumgart et al. Aging Cell 2015).
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	For animal experiment (in vivo proteasome inhibitor), sample size was determined on the basis of the results of a pilot experiment.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No sample was excluded from the analysis
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	For animal experiment (in vivo proteasome inhibitor), animals were randomly allocated to the different experimental groups.
For animal studies, include a statement about randomization even if no randomization was used.	See above
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	For animal experiment (in vivo proteasome inhibitor), animals were randomly allocated to the different experimental groups. No blinding of the investigator was performed when analysing the data.
4.b. For animal studies, include a statement about blinding even if no blinding was done	See above
5. For every figure, are statistical tests justified as appropriate?	Statistical tests for each figure are stated in the figure legend. Descriptive statistics, such as assessment of normal distribution and coefficient of variation, were performed for each dataset, in order to choose adequate statistical methods. For example, for figures such as 4E/F, where three groups were compared to one another, normal distribution of the dataset was checked by multiple tests (Anderson-Darling, D'Agostino & Pearson, Shapiro-Wilk, Kolmogorov-Smirnov tests). Equality of variance was assessed by either Brown-Forsythe and/or Bartlett's tests on performing one-way ANOVA. Multiple comparison tests were also applied and stated in the figure legend.
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Is the variance similar between the groups that are being statistically compared?	See above
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C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	These information are reported in the Material and Methods section
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	No cell line was used in this study.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	List of animals used is reported in Table EV1. Housing details are provided in Material and Methods.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All the experiments were authorised by the local authorities and performed according to the relevant legislation. Protocol approval numbers are reported in the Material and Methods.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We confirm compliance with ARRIVE guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	na
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	na
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	na
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	na
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	na
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	na
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	na

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	Accession codes are reported in the Material & Methods under the Data Availability section.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	na
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G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	na
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